Segregation of Clock and Non-Clock Regulatory Functions of REV-ERB

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The molecular clock is a master controller of circadian cellular processes that affect growth, metabolic homeostasis, and behavior. A report in *Science* by Zhang et al. (2015) redefines our understanding of how Rev-erbα acts as an internal feedback inhibitor that modulates activity of the core clock while simultaneously regulating tissue-specific metabolic processes.

The molecular clock is composed of a transcriptional-translational feedback loop. Heterodimers of the transcription factors BMAL1 (brain and muscle ARNT-like protein 1) and CLOCK (circadian locomotor output cycles kaput) or NPAS2 (neuronal PAS domain containing protein 2) stimulate gene expression of *period* (*Per1,2,3*) and *cryptochrome* (*Cry1,2*). PER/CRY heterodimers then inhibit the activity of BMAL1–CLOCK/NPAS2 leading to a self-sustaining oscillation in gene expression. The nuclear receptors (NRs) Rev-erb (Rev-erbα, Rev-erbβ) and retinoic acid receptor-like orphan receptor (RORα, RORβ, and RORγ) directly regulate *Bmal1* gene expression. Rev-erbs and RORs recognize and compete for the same "response elements" in the genome, including the one found in the *Bmal1* promoter. Rev-erbs are transcriptional repressors, while RORs are transcriptional activators. Rev-erbs and RORs are expressed in a circadian fashion antiphase to one another, contributing to the circadian pattern of clock gene expression. Physiologically relevant ligands for both of these NR classes have been identified, implying that they also function as sensors of nutrient flux and/or metabolic state (Kojetin and Burris, 2014). Rev-erbs serve as receptors for heme (Raghuram et al., 2007; Yin et al., 2007), whereas RORα and RORγ display high affinity for various oxysterols (Jin et al., 2010; Kallen et al., 2002; Wang et al., 2010).

Beyond the clock, Rev-erbs and RORs regulate expression of genes involved in immune function, behavior, muscle metabolism, and lipid and glucose homeostasis. A recent publication suggests that Rev-erb may employ distinct regulatory mechanisms with regard to regulation of clock genes versus other tissue-specific non-clock genes (Zhang et al., 2015). Unexpectedly, comparing Rev-erbα cistromes in the brain (~20,000 binding sites), liver (~9,000), and white adipose tissue (~8,500) revealed remarkably little overlap in receptor binding sites. Only 183 sites were common to all three tissues, typically near clock genes including *Bmal1*, *Cry1*, *Npas2*, *Nr1d1*, and *E4pb4*, containing classically defined Rev-erb DNA response elements (ROREs and RevDR2s). However, the tissue-specific sites did not typically contain ROREs or RevDR2s. In fact, these sites were enriched for response elements for other transcription factors specific for each tissue examined. For example, Rev-erb binding sites in the liver were heavily enriched for HNF4A and HNF6 binding sites, while in the fat CEBP response elements were enriched. In the liver, RORs (RORα and RORγ) competed for common Rev-erb binding sites in clock genes, but not in Rev-erbα tissue-selective binding sites.

The authors hypothesized that Rev-erb might be regulating many genes independent of its DNA binding domain. This was tested using a Rev-erbα knockout mouse that expresses a mutated Rev-erbα protein lacking a DNA binding domain (DBD^m mice). In mice only expressing the DBD^m Rev-erb, Rev-erb binding sites were dramatically reduced at the common sites (clock sites); however, the binding signal at the tissue-selective sites was maintained. This demonstrates that there are two mechanisms by which Rev-erb can regulate genes—DBD dependent and DBD independent. Remarkably, the DBD-independent mechanism may be the predominant mechanism, perhaps involving tethering to other factors.

Liver DBD-independent Rev-erb sites were enriched for HNF6 DNA response elements, and the group demonstrated that these sites were indeed bound by HNF6. Furthermore, simultaneous binding of Rev-erbα and HNF6 at DBD-independent sites was observed, but not DBD-dependent sites. Physiological importance of the DBD-independent sites was supported by comparison of gene expression and metabolic phenotype of Rev-erbα DBD^m and Rev-erbα knockout mice. Rev-erb-regulated genes involved in hepatic lipid metabolism are predominantly regulated by the DBD-independent mechanism, since they were de-repressed in Rev-erbα knockout mice, which also exhibited hepatic steatosis, but were expressed normally in Rev-erbα DBD^m mice that were not steatotic.

Several NRs have been shown to exert transcriptional regulatory effects via indirect tethering. However, the finding that the vast majority of the Rev-erb binding sites do not require the DBD was clearly not expected. Segregating function in terms of DBD-dependent versus -independent sites is also important. The DBD-dependent sites focus primarily on clock regulation, while DBD-independent sites focus on tissue-selective functions. This separates circadian regulation that is found in all cell types, where competition with RORs is an important component of the pathway, from the maintenance of circadian oscillations in expression of tissue-selective Rev-erb target genes. One role of the core clock is to maintain high-amplitude circadian oscillations in Rev-erb expression (*Rev-erba* is one of the common DBD-dependent genes itself). DBD-independent genes may therefore be considered

"clock-controlled genes" (CCGs). The "tethering" mechanism also allows for "modular" flexibility between cell types expressing different anchor proteins. The RORs display a similar profile to Rev-erbs in terms of a very distinctive function regulating the clock, but additional specific roles in the regulation of development, immune function, etc., exist. It is possible that RORs operate with a similar segregation of function, but future work will be required to address this possibility.

We have been intrigued by the distinct activities of drugs targeting these two classes of NRs. If the predominant mechanism of action of these receptors were via direct DNA binding via recognition of a RORE/RevDR2, then targeting them should have similar outcomes with Rev-erb activators acting similar to ROR inhibitors. However, this has not been observed in many cases. For example, Rev-erb agonists (Banerjee et al., 2014), but not RORα/γ inverse agonists (T.P.B., unpublished data), are anxiolytic and induce wakefulness. The research by Zhang et al. (2015) suggests that most genes targeted by Rev-erb are regulated independently of the DBD and are thus also regulated independently of competition from RORs. Considerable differences between the actions of these drugs are therefore expected. Zhang et al. (2015) also suggest that, due to the distinct regulatory mechanisms, it may be possible to pharmacologically target DBD-independent Rev-erb pathways while sparing the DBD-dependent pathways, providing for a drug avoiding general effects on the circadian clock. This would not be a trivial undertaking given that we typically target Rev-erb's ligand binding domain. It is also unclear whether one could modulate DBP-dependent and -independent pathways independently with our current understanding of NR drug development. Finally, it is fascinating to speculate that we could pharmacologically target specific tissues using synthetic Rev-erb ligands designed to modulate tissue- specific Rev-erb/tethered partner complexes.

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REFERENCES

Banerjee, S., Wang, Y., Solt, L.A., Griffett, K., Kazantzis, M., Amador, A., El-Gendy, B.M., Huitron-Resendiz, S., Roberts, A.J., Shin, Y., et al. (2014). Nat. Commun. *5*, 5759.

Jin, L., Martynowski, D., Zheng, S., Wada, T., Xie, W., and Li, Y. (2010). Mol. Endocrinol. *24*, 923–929.

Kallen, J.A., Schlaeppi, J.M., Bitsch, F., Geisse, S., Geiser, M., Delhon, I., and Fournier, B. (2002). Structure *10*, 1697–1707.

Kojetin, D.J., and Burris, T.P. (2014). Nat. Rev. Drug Discov. *13*, 197–216.

Raghuram, S., Stayrook, K.R., Huang, P., Rogers, P.M., Nosie, A.K., McClure, D.B., Burris, L.L., Khorasanizadeh, S., Burris, T.P., and Rastinejad, F. (2007). Nat. Struct. Mol. Biol. *14*, 1207–1213.

Wang, Y., Kumar, N., Solt, L.A., Richardson, T.I., Helvering, L.M., Crumbley, C., Garcia-Ordonez, R.D., Stayrook, K.R., Zhang, X., Novick, S., et al. (2010). J. Biol. Chem. *285*, 5013–5025.

Yin, L., Wu, N., Curtin, J.C., Qatanani, M., Szwergold, N.R., Reid, R.A., Waitt, G.M., Parks, D.J., Pearce, K.H., Wisely, G.B., and Lazar, M.A. (2007). Science *318*, 1786–1789.

Zhang, Y., Fang, B., Emmett, M.J., Damle, M., Sun, Z., Feng, D., Armour, S.M., Remsberg, J.R., Jager, J., Soccio, R.E., et al. (2015). Science *348*, 1488–1492.

A New Hydroxy Metabolite of 2-Oxoglutarate Regulates Metabolism in Hypoxia

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Two articles in this issue (Intlekofer et al., 2015; Oldham et al., 2015) show a new metabolic pathway regulated by hypoxia, but independently of HIF1 or HIF2. L-2-hydroxyglutarate, produced in hypoxia by malate dehydrogenases and LDHA, is a potent inhibitor of KDM4C, and through redox stress reduces glycolysis.

A key step in the Krebs cycle is the conversion of isocitrate to 2-oxoglutarate, and thereafter to succinate. Mutations in isocitrate dehydogenases *IDH1* and *IDH2* result in conversion of 2-oxoglutarate (also known as alpha-ketoglutarate) to D(R)-2-hydroxyglutarate, an oncometabolite that can inhibit enzymes that use 2-oxoglutarate as a cofactor with oxygen (Losman and Kaelin, 2013). These are dioxygenases, which include TET1 and TET2, enzymes that modify 5-methylcytosine residues in DNA and hence gene transcription (Figure 1).

Two papers published in this issue (Oldham et al., 2015; Intlekofer et al., 2015) describe another metabolite, a normal product of 2-oxoglutarate metabolism, the L(S)-2-hydroxyglutarate isomer. This metabolite was previously noted as a product of IDH in hypoxia, but its role was unknown. These papers present evidence of increased production of L-2-hydroxyglutarate in hypoxia. The